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Georgia Institute of Technology

Environmental Engineering Program
School of Civil and Environmental Engineering
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30 September 2000

Dr. Ratib KARAM
ERDA Neely Research Center
Georgia Tech
Atlanta, GA

Subject: Southern Sector Seepline Research Project (E-20 E84)

Dear Dr. KARAM,

Enclosed is a final report, "Phytoremediation of TCE and PCE in the Southern Sector of SRS", for the subject ERDA project.

This report and project is a collaborative effort of Dr. Robin Brigmon of SRTC, and members and collaborators on his staff, and me and my staff at GA Tech. The research activity is also in collaboration with DOE staff and Environmental Restoration Division - SRTC staff. This report is currently under review and comment by ERD.

Please provide DOE and ERDA personnel with a copy of this report. If additional copies are required, please let me know. The report at this time is not available as an electronic file due to its review status and size.

Your assistance with the appropriate distribution of this report is appreciated, as will be your efforts in assisting me with continuation of this collaborative effort with DOE.

Sincerely,

F. Michael Saunders
Professor

Cc: D. Aiken, OCA-Reports Coordinator, C. D'Urbano, D. Bridges

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FY00 Phytoremediation of Trichloroethylene and Perchloroethylene in the Southern Sector of SRS

Interim Report

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LIST OF KEY WORDS
Phytoremediation, bioremediation, perchloroethylene, trichloroethylene, and groundwater.

September 2000

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Executive Summary

This study addresses the fate of volatile organic contaminants (VOCs) in an experiment that simulates a vegetated seepline supplied with trichloroethylene (TCE) and perchloroethylene (PCE) - contaminated groundwater. The primary object is to determine if the trees take up the TCE and PCE, accumulate it, or transform it. Experimental focus is on removal of VOCs from the groundwater by phytoremediation or associated soil microorganisms. The removal of chlorinated ethenes by phytoremediation and monitored natural attenuation (MNA) using these technologies at the field scale is ongoing.

In October 1999, SRS initiated a pilot-scale phytoremediation study to support remediation of chlorinated ethenes in the Southern Sector of A/M Area. The project has concentrated on groundwater above the seepline at Tim's Branch. The field research has the objective of determining the role of plants and soil for *in situ* bioremediation of the VOCs TCE and PCE under specific site conditions. Well MSB 88 was selected as the groundwater supply because of its VOC concentrations (TCE 188 ppb, PCE 55 ppb) and proximity to the seepline. Three phytoreactors were deployed with soil from the seepline. Phytoreactor 1 was planted with loblolly pines (*Pinus taeda*) native to SRS, phytoreactor 2 was planted with hybrid poplars (*Trichocarpa* X *deltoides*), and phytoreactor 3 was left nonvegetated as a soil control to evaluate monitored natural attenuation (MNA) of TCE and PCE in the treatability study.

The test cells were supplied with a continuous flow of contaminated groundwater from well MSB 88. Preliminary results from the study show reduction in groundwater VOCs and suggest removal of the contaminants by the trees and soils at the concentrations tested are possible. Results to date demonstrate that the loblolly pine phytoreactors can remove up to 90 % of the TCE and 80 % of the PCE. The hybrid poplar phytoreactors demonstrated up to 100% removal of the groundwater contaminants. No detectable amounts of these VOCs were found in transrespiration, soil volatilization, or soil core testing. Microbial activity in the phytoreactors and seepline soils is under investigation. We are using anaerobic microcosms for assessing biotransformation of TCE and PCE to degradation products. Microcosms are currently under incubation and analysis of biodegradation is in process.

The ongoing research project is part of a multi-year field study of SRS seepline-soil systems maintained under saturated conditions. The primary focus is on determining the roles of (i) trees; (ii) seepline groundcover (iii) soil microbial communities, as well as geochemical and surface-volatilization processes, in determining the fate of TCE and PCE in contaminated groundwater that flows through surface seepline areas. Previous observations indicated that biogeochemical processes of plants are seasonal, as are seepline flows by previous observations. Therefore, FY00 represented an initial acclimation phase for soil and plant systems and will facilitate examination of seepline phyto- and bioactivity in subsequent growth season in FY01. Initial results indicate that phytoremediation and MNA have considerable potential for the removal of TCE and PCE in the Tim's Branch flood plain and seepline.

BACKGROUND

It has been estimated that over 13 million pounds of chlorinated degreasing solvents, including trichloroethylene (TCE) and perchloroethylene (PCE) were used at SRS during reactor operations. Although much of the waste volume was reduced by evaporation, over 3 million pounds, including 317,000 pounds of TCE, were discharged to the M-Area Settling Basin and the A-014 outfall. The M-Area Settling Basin and A-014 outfall were unlined and much of these solvents seeped into the subsurface contaminating groundwater. The associated groundwater zones in A/M Area (i.e. M-Area and Lost Lake Aquifers) discharge to seeplines adjacent to Tims Branch and Upper Three Runs Creek (WSRC-TR-99-00113). As part of the ongoing compliance and research activities at SRS, evaluations of the nature and extent of groundwater contamination in the A/M-Area are ongoing in the Southern Sector, primarily between recirculation wells and the seepline area. Based on the local hydrogeology and topography, it was predicted that VOC contaminated groundwater would emerge as surface water along a seepline region in the Southern Sector of the A/M-Area. The seepline is presently heavily covered with a variety of vegetation. This area lends itself to the potential of phytoremediation with species known to degrade VOCs and monitored natural attenuation (MNA).

Previous research at SRS has demonstrated the potential for phytoremediation of chlorinated ethenes. A recent investigation of a shallow VOC contaminant plume at the SRS TNX flood plain demonstrated that bald cypress (*Taxodium distichum*), tupelo (*Nyssa aquatica*) and loblolly pine (*Pinus taeda)* contained significantly higher levels of chlorinated ethenes than adjacent oak (*Quercus* spp.) and sweet gum (*Liquidambar stryaciflua*) trees in the same area (Vroblesky, Nietch, and Morris, 1999). Walton and Anderson (1990) previously observed accelerated microbial degradation of TCE in slurries of rhizosphere soil and mineralization of TCE in whole plant systems collected from samples at a former SRS solvent disposal site, the Miscellaneous Chemical Basin (MCB). Two species where enhanced microbial degradation of TCE was observed at the SRS were a legume, *Lespedeza cuneata* and loblolly pine, *Pinus taeda* (Anderson and Walton, 1995).

The extent to which rhizosphere VOC remediation varies among soils in this area is uncertain. However, a better understanding of such variability is necessary since rhizosphere responses to seasonal changes including plant succession, rainfall, and temperature can significantly influence potential VOC bioremediation. A microcosm study undertaken to estimate the potential of Southern Sector rhizosphere soils along the seepline to naturally attenuate TCE demonstrated that sorption to soil was the dominant mechanism with as much as 90% TCE removal (Brigmon et al., 1998). A limited amount of TCE aerobic biodegradation and anaerobic reductive dechlorination was observed through the appearance of *cis*-1, 2-dichloroethylene (c-DCE), and *trans*-1, 2-dichloroethylene (t-DCE) in microcosm tests. Soils from vegetated areas mineralized TCE several times greater than soils from adjacent non-vegetated areas (Walton and Anderson, 1990). It has been suggested that a possible mechanism for the enhanced microbial mineralization of TCE in the *L. cuneata* rhizosphere soil is excretion of phenolic compounds in root exudates. Since phenol is a known inducer of toluene monooxygenase, an enzyme responsible for degradation of TCE, the natural plant exudates could play a role in biodegradation of TCE in the rhizosphere (Anderson, Guthrie, and Walton, 1993). The microbial data collected in this study through

the BIOLOG system that tests for aromatic substrate activity could demonstrate this potential. Select plants including hybrid poplars are capable of TCE metabolism and transformation (Newman et al., 1997; Schnabel et al., 1997). Selection of the appropriate plant species can be critical to the success of phytoremediation technology. The two tree species selected for this study based on their phytoremediation potential were the loblolly pine, *L. cuneata* and a hybrid poplar, *Trichocarpa* X *deltoides*. In this project, both soil/microbial and tree activity as pertaining to VOC attenuation is being monitored.

One of the primary functions of root exudates is to mobilize inorganic nutrients required by the plant. Exudates also contain natural chelating agents (citric, acetic, and other organic acids) that make the ions of both nutrients and contaminants more mobile in the soil. Exudates may also include enzymes, such as nitroreductases, dehalogenases, and laccases. These enzymes have important natural functions, but may also degrade organic contaminants that contain nitro groups or halogenated compounds (Fliermans et al., 1988). Some rhizosphere microorganisms secrete plant hormones that increase root growth, and thereby the secretion of root exudates that contain metabolites used by the bacteria including proteins and carbohydrates. Exudation of organics by plant roots and turnover of organic root biomass has also been found to increase the TCE sorption capacity of soil (Schnabel et al., 1997). The microbial ecology of soil associated with bioremediation in mycorrhizal roots such as pine has not been well characterized even though this environment forms a large habitat and provides extensive surface area for bacterial colonization. It was previously observed that the rhizosphere soils in the SRS MCB contained higher quantities of potential TCE-degrading bacteria samples than the Southern Sector soil samples that have not been exposed to chlorinated-solvent contamination (Brigmon et al., 199). Nichols et al. (1997) has previously demonstrated that higher microbial populations are present in organic-contaminated rhizosphere soils as compared to non-contaminated rhizosphere soils. These microbial data from this year emphasize the heterogeneous nature of rhizosphere plant-microbial interactions and provides a foundation for more focused microbial studies this next year.

A better understanding of the potential mechanisms involved for enhanced biodegradation in the root zone and the interaction between plants, microorganisms, and contaminants may be useful in phytoremediation applications by helping in the plant selection process. This information could lead to improved land management practices for phytoremediation applications including selection of plants, soil amendments, and irrigation systems. Future work based on these techniques could be used to determine phytoremediation potential in response to TCE/PCE-contaminated groundwater seepage through the rhizosphere.

The techniques describe herein in conjunction with other applications should provide tools for screening plant species and soils for phytoremediation and MNA activity. Application of phytoremediation should provide significant advantages over conventional remediation techniques for chlorinated ethene-contaminated groundwater. The metabolic actions of the plants and soils in combination with physical reductions of VOCs by volatilization and dilution will enable active remediation at the rhizosphere of the seepline.

ACTIVITIES

Field Treatability Test. After initial site selection (Well MSB 88), an evaluation of Southern Sector soil was undertaken. The area selected was based on the proposed location identified by the Environmental Restoration Department and the well VOC concentrations. The soil in the area of MSB 88 is very sandy with red clay not representative of the seepline. Therefore, soils had to be brought to the study site from the seepline. Soils above (to 0.5 m depth below surface) and below the rhizosphere (0.5-1 m depth below surface) were collected in the vicinity of Well MSB 50 (located in the vicinity of the seepline area for the study. The Georgia Institute of Technology (GT) set up soil columns on 5-28-99 for initial flow and soil-permeability tests. Hydraulic permeability and porosity measurements were made to assist in soil characterization and assessing cell design and soil placement in the phytoreactors. On 9-28-99, the double insulated boxes (72"x 48"x30") (Bonar Inc., Atlanta, GA) were brought to the site for set up. Initial startup testing of the boxes began with groundwater from MSB-88 B that had low (5 ppb) VOC concentrations. Figure 1 shows a diagram of the project field deployment configuration. Figure 2 illustrates the phytoreactor and the set up process. In January 2000 permission was received to use Well MSB 88C that had concentrations in the 120-ppb range for TCE and 60 ppb range for PCE. On 3-20-00, all boxes were placed in operation receiving contaminated groundwater.

The boxes (phytoreactors) were developed with an upflow pattern of groundwater flow. Groundwater from MSB-88C is pumped into a 1000 gal polypropylene storage tank that supplies the phytoreactors though a gravity-fed system. A 3-in. layer of gravel in the bottom of the cells supports a 2-line influent-distribution system in the bottom of each cell. The gravel layer was then covered with 20 in. of seepline soil. Two separate effluent collection systems were included in each cell. The effluent collection lines are located 10 in. and 18in. (i.e., immediately below the soil surface) above the influent lines and are parallel to them. This flow pattern allows for simulation of the upflow of groundwater through the seepline soils and the collection and removal or the groundwater below the root zone of the plants. The effluent collection system at the 10-in. depth is the one being used in all cells and provides a 10-in. saturated flow zone and a 10-in. vadose zone for the cells. Three phytoreactors were set up for the project. Loblolly pine (*L. cuneata*) was planted in Box 1, the hybrid poplar (*Trichocarpa* X *deltoides*) in Box 2, and Box 3 contained only seepline soil as a non-vegetated control. A 1000-gallon steel tank is used for effluent collection downhill from the site. A surface drain system is under construction to allow for surface runoff and a surface–litter layer will be placed in each cell to minimize the development of a surface-clogging biomass.

Collection of Samples. Sampling groundwater from the boxes for chemical and microbial analysis began on March 30, 2000. During April 2000, the boxes were sampled weekly for groundwater influent and effluent microbial activity, VOCs, and ion analysis. Thereafter, influent and effluent groundwater and soils from the boxes were sampled monthly.

Analyses

Flow measurements. Groundwater supplying the boxes was measured with flowmeters interfaced with a datalogger powered by a solar cell. While SRTC assisted in set up of the system, GT weekly monitored the flow data. The influent flow rate for Cells 1, 2, and 3 was collected using a digital flow meter and a Campbell Scientific datalogger. The flow rates for each cell were logged every fifteen minutes. This fifteen minute value represented an average flow rate over that particular time period.

Gas Chromatography. Samples were taken from groundwater influent and effluent for VOC analysis. Soil samples were collected by hand auger from four locations in each box, two shallow (.5 m), and two deep (1 m). Each soil sample was collected with a modified plastic syringe and placed directly into a 20 mL glass vial with 5 mL deionized water and immediately sealed for subsequent VOC analysis. Chlorinated ethene analysis was performed on samples in the sealed glass vial using headspace gas chromatography (GC). The headspace GC method minimizes sample handling and preparation and measures the bulk (sorbed, dissolved, vapor, and NAPL) TCE content of the sample. Samples were analyzed using a Hewlett-Packard 5890 gas GC equipped with a flame ionization detector (FID) and a 60-m SPB1™ column (0.75-mm ID, 1-μm thick; Supelco, Bellefonte, PA). Transrespiration and soil volatilization gas samples were collected in the field by the method described by Newman et al. (1997). Manual injections of samples from Tedlar gas bag samples from transrespiration and soil volatilization measurements were made with a 250-μL gastight syringe (Precision Scientific, Baton Rouge) (Newman et al., 1997).

Ion Chromatography. Chloride, nitrite, nitrate, phosphate, and sulfate groundwater concentrations were measured with a Dionex QIC-2 ion chromatograph equipped with a conductivity detector, and a 250-mm Dionex IonPac Fast Anion Analytical column (4-mm ID, 16-μm bead; Dionex Corp., Sunnyvale, CA), operated at ambient temperatures. A 2 mM sodium carbonate/0.75 mM sodium bicarbonate buffer solution was used as the eluent (2 mL/min). Samples were taken from the supernatant of a solution prepared from groundwater or 1 g of dry soil (dried at 121◦C for 24 hours) and 5-mL of deionized water vortexed for 1 minute then centrifuged for 5 minutes at 2500 rpm.

Microbial densities. Comprehensive analysis of specific microbial species and populations, and characterization of the metabolic potential of whole microbial communities, can be an effective tool to predict the potential, or monitor the activity of microorganisms in reducing and/or removing harmful groundwater contaminants. Groundwater samples were collected in sterile 50-mL centrifuge tubes and transported to the lab for immediate processing. Total microbial population densities in phytoreactor influent and effluent groundwater and soils were determined by the Acridine Orange Direct Count (AODC) Method (Balkwill, 1989). The viable microbial population densities of aerobic and facultative heterotrophic bacteria in groundwater and soils were determined using spread plate techniques. Low concentrations (1%) of Peptone-Trypticase-Yeast extract-Glucose (PTYG) media was used (Balkwill, 1989). Community-level physiological analysis using BIOLOG GN2 plates indicates the utilization rate of 95 carbon sources by microorganisms in the groundwater. Individual substrate utilization patterns of

groundwater microbial communities were obtained with Biolog GN2 plates from each groundwater sample. Groundwater (150 μl) samples was used for direct inoculation of the plates. Autoclaved deionized water is used as a control. All plates were incubated at room temperature and the absorbance (590 nm) of the wells recorded after 1 week. The color intensity of Biolog GN2 plates was expressed and calculated as the mean of the 95-absorbance values corrected for the background control.

Microcosm Studies. Microcosm tests were set up at GT to assess the microbial activity and ability of phytoreactors and seepline soils to transform PCE, TCE, and typical degradation products of TCE and PCE. Anaerobic microcosms are being used to assess activity for transformation of TCE and PCE to degradation products, as well as the presence of bacterial populations indicative of other favorable bioprocesses (e.g., halorespiration and methanogenesis). Microcosms were supplemented with several primary substrates, including acetate, lactate and Hydrogen (H_2).

Microbial Respiration. Metabolic chambers were set up with a respirometer (Columbus Instruments Inc., Columbus, OH) to measure soil microbial respiration in the phytoreactors. This method measures the rates of soil oxygen consumption and carbon dioxide production. These measurements were made in August, 2000, when the trees were fully developed. Two soil samples were taken from each box with a stainless steel hand auger. A shallow sample was taken from the top 0.5 m of soil and a deep sample was taken from the bottom of the box in the saturated zone.

RESULTS and DISCUSSION

Flow measurements. Groundwater supplying the boxes has been measured with flowmeters interfaced with a datalogger powered by solar cells. While all boxes were set up with an influent of 20 mL groundwater per minute(~7.6 gal/d), the rate constantly changes as a result of soil settling, weather conditions, plant growth and root development (particularly in Box 2), and changes in the supply system (i.e. supply tank water levels). Included are plots of data from Boxes 1, 2, and 3 demonstrating the variability in the flow data (Figure 3 a-c). The peaks in flow are when the supply tank is filled. The influent supply and effluent system will have major changes including raising the height of the supply tank, increasing the diameter of influent and effluent system piping, and drains on the soil surfaces in the next month to improve the groundwater flow rate continuity. Figure 10 demonstrates data from a groundwater effluent flow study from all three boxes from 8/9/00 to 8/17/00. In comparing the groundwater influent flow rates from the three boxes (Figure 3) to effluent flow rates (Figure 4) it is of interest that Box 2 with the poplars appeared to have the highest influent groundwater input and yet the lowest effluent measured.

Removal of TCE and PCE. When filling the supply tank (Figures 1 & 2) it was found that Well MSB 88 has consistent VOC concentrations (TCE 188 ppb, PCE 55 ppb). By the time the test cells or phytoreactors filled with seepline soils actually receive the continuous flow of contaminated groundwater the concentration averages around 46 ppb TCE and 48 ppb PCE. The concentration of TCE and PCE in both phytoreactor influent and effluent groundwater is shown in Figures 5 and 6,

respectively. All three boxes show a reduction in both TCE and PCE in the effluent compared to the influent. Box 2 (Hybrid Poplar) has shown no detectable PCE or TCE in the effluent groundwater for June and July 2000 sampling events, indicating total removal. In July, the first samples were taken for soil volatilization and plant transrespiration. Manual injections from Tedlar gas bag samples for transrespiration and soil volatilization measurements were made with a 250-μL gastight syringe (Precision Scientific, Baton Rouge) as described by Newman et al., (1997). No detectable TCE or PCE (<5ppb) was found in soil volatilization samples from any of the boxes. All measurements from the pine and poplar showed no detectable amounts of TCE and PCE being respired. These measurements will be repeated in August, 2000.

There appear to be significantly larger ($P < 0.05$) TCE and PCE concentrations in the influent than in the effluent in every box (1, 2, and 3). However, there was no significant correlation between the Influent/Effluent difference and any of the covariates, viz., cumulative mean daily temperature, cumulative daily A/M-area rainfall, or the mean of the day's barometric pressure for the given sampling date. In other words, these covariates were not helpful in partitioning out any variability in the response measure of Influent/Effluent difference for either TCE or PCE. The same is true for the cumulative maximum daily temperature, cumulative maximum SRS rainfall, or the maximum of the day's barometric pressure. Both the common logarithm and the cube root transformations were also used on the Influent/Effluent measurements to see if differences in the transformed variables were correlated with the covariates. In no instance, however, was there a statistically significant correlation between a transformed response variable difference and any of the covariates.

Although poplars (Box 2) appeared to be the most effective treatment in recent months (Figures 5 & 6), no statistically significant difference existed between any pair of boxes when comparing the average Influent/Effluent groundwater PCE/TCE difference among boxes. This suggests that the treatment assigned to each box was as effective in the remediation of both TCE and PCE as any other box. In order for this interpretation to be convincing, we must show that the Influent/Effluent difference was not due in large measure to the groundwater distribution system. We must show that the delivery system was not simply overwhelming whatever treatment differences there might have been between pairs of boxes.

Plant tissues (roots, stems, and leaves) from the pine and poplar have recently been taken from the phytoreactors for analysis of PCE, TCE and potential metabolic breakdown products including trichloroacetic acid (TCA), and dichloroacetic acid (DCA). This ongoing analysis will provide useful information on the fate of the chlorinated ethenes in the system.

Ion Chromatography. Table 1 shows the influent and effluent groundwater chloride, nitrite, nitrate, phosphate, and sulfate concentrations. The composite water flows, and resulting flow of soluble ions, for the cells include influent groundwater, influent rain water, subsurface discharge of groundwater and evaporative losses at the soil surface (cells 1, 2 &3) and evapotranspiration by plants (in cells 1 & 2). In addition, the soil placed in the cells contained pore water moisture with dissolved minerals, as well as

minerals sorbed to soil surfaces. These flows and sources need to be considered in the assessment of the ion data to date.

Chloride ion should be conservative in the cells and, after an initial perturbation in March for the effluent, the influent and effluent data for chloride appear to be similar. The initial phosphate concentration data in March may represent cross contamination of the influent tank; but thereafter, influent and effluent phosphate concentrations were at trace levels.

Sulfate levels in the effluent of the cells appear to be elevated, relative to the influent in all cases. Sulfate elution from the soils would appear to be the most plausible assessment of this increase, although it is possible that there is sulfide oxidation taking place in the saturated zone. Nitrogen species in the system are nitrate and nitrite. Nitrate concentrations decrease through the cells, relative to the influent, and nitrite appears in the effluent despite being at non-detect levels in the influent. The transformations of nitrate and nitrite are indicative of (i) plant uptake of nitrogen species and (ii) denitrification by soil microbes. Plants will use nitrate and a primary source of nitrogen and this could indicate the growth taking place in box 1 & 2. Box 3 has no trees, so the changes in this cell would be microbially based. The occasional presence of nitrite in effluents would indicate that anaerobic respiration was in process and that nitrite conversion to nitrite and ultimately to nitrogen (N_2) was occurring in the cells. These responses need to be further examined in the coming year. Finally, the issue of nutrient addition is supported by these nitrogen and phosphorus data (i.e., phosphorus and nitrogen are at low levels and supplementation is warranted). No significant difference between treatments across time was found at this time. The addition of a slow-release fertilizer to the surface soils will be implemented this next year.

Microbial densities. In all cases the total microbial densities as measured by AODC were higher in the effluent from the phytoreactor groundwater than the influent groundwater (Figure 7 a-c). The source of the influent bacteria is the result of bacteria from influent groundwater, and microbial growth in influent tank, filter, and associated supply lines. Bacteria in the effluent groundwater are from soils placed in the cells, influent groundwater, and environmental origin (air, rain, etc.), as the phytoreactors are open systems. The viable microbial population densities of aerobic and facultative heterotrophic bacteria in groundwater as measured by colony forming units/mL (CFU/mL) were more variable (Figure 8 a-c) than the AODC density-based data. The viable influent groundwater bacteria were generally in lower concentrations as compared to effluent. Box 2 containing the poplar trees appeared to have lower concentrations of viable bacteria in the effluent groundwater relative to the other two boxes (Figure 8b). Analysis of the BIOLOG data for substrate utilization is ongoing. When comparing influent and effluent CFU/mL of all three boxes, there appeared to be a statistically significant and lower plate count in Box 2 containing the poplars compared to the other boxes (Figures 8b and 9).

Microbial data from testing groundwater use of BIOLOG for total amount of substrate utilization demonstrated a similar trend for all three phytoreactors (Figure 10). In Figure 6 the difference in the number of positive BIOLOG positives represents the delta between total substrates utilized (of 95

possible) in effluent vs. influent groundwater for each box. Further analysis of substrate utilization by category (i.e. aromatics vs. carbohydrates) is underway.

Microcosm Studies. Microbial activity in the phytoreactors and seepline soils has been investigated and is described above. Anaerobic microcosms are being employed to assess activity for transformation of TCE and PCE to degradation products.

Soil samples were obtained from the seepline and the phytoreactor cells at the site in May, 2000. Soil samples have been handled in an anaerobic glove box at all times in the laboratory and kept under refrigeration at 4∘C prior to analysis. Soil samples were labeled in the following way.

Label	Description	Source
SED 1	Mixture of 3 samples of soil from phytoreactors	Three independent samples taken as soil cores (2 cores per reactor) from phytoreactors 1, 2, 3
SED 2	Seepline soil	Seepline area near Steeds Pond and at same location of site for soil in the reactor cells

Anaerobic microcosms were established to evaluate the potential for indigenous microorganisms to dechlorinate PCE, TCE, dichloroethene (cis-DCE), and vinyl chloride (VC) to subsequent end-products. Microcosms were established in 20-mL vials. Each microcosm contains 2 grams of wet sediment material, 9 mL of phosphate buffered ground water containing a designated electron donor, and 100 μL of hexadecane containing the chlorinated compound, i.e. PCE, TCE, or cis-DCE. Vinyl chloride was added to designated microcosms in gaseous form (i.e., not with hexadecane). The electron donors used were lactate, acetate, or hydrogen (H_2). Acetate and lactate were added at a concentration of 2mM. In the microcosms containing hydrogen (H_2) as electron donor, 3mL of hydrogen gas were added to each microcosm. Resazurin was used as a redox indicator. This dye stays colorless when reduced and becomes pink when oxidized, thus quickly indicating any oxygen contamination of the microcosm. The electron acceptors (chlorinated compounds) used were PCE, TCE, cis-DCE, and VC.

The contaminant concentrations utilized were the following:

PCE at 1.25 μL/100 μl hexadecane ~12μmol in system
TCE at 0.8 μL/100 μl hexadecane ~ 9μmol in system
cis-DCE at 0.3 μL/100 μl hexadecane ~ 4μmol in system
VC at 0.2 mL/ vial ~8μmol in system

Microcosms with SED 1 and SED 2 were established during the last two weeks of May 2000. After the microcosms were established, they were sealed with Teflon-lined butyl rubber stoppers and incubated without agitation at 25∘C. Headspace samples (0.1mL) are analyzed monthly (HP 6890 gas chromatograph with a FID detector) to monitor dechlorination activity. Controls include vials containing no electron donor, no electron acceptor and autoclaved (killed) controls. Table 2 summarizes the microcosms established with SED1 and SED 2.Initially, only the PCE containing microcosms were analyzed, because when dechlorination occurs under anaerobic conditions, the more highly chlorinated compounds are more rapidly dechlorinated. A response of no dechlorination in the PCE containing microcosms indicates that most probably dechlorination has not taken place in the rest of the microcosms. After 1, 2, and 3 months all TCE and PCE-containing samples established with SED 1 and SED 2 were analyzed and no intermediates were detected. These data indicate that there are no resident microbial populations transforming PCE or TCE in the cell soils. This response is compatible with the porewater and effluent groundwater data indicating, in general, an oxidizing environment within the saturated soil zone.

For the soils from the phytoreactors, i.e., SED1, no dechlorination products were found. However, methane production was detected in some of the microcosms, as shown in Table 2. Table 3 shows that all the SED1 test microcosms, except the TCE microcosms, have some methanogenic activity. Microcosms with SED 2 showed no dechlorination products, and no methane production was observed. These data indicate the potential for the development of methanogenic population, but do not indicate that methanogens are present in the soils of cells 1, 2 or 3 at any significant levels. In fact, the seepline soils (SED 02) did not demonstrate methanogenesis in the microcosm studies. The microcosm analyses will continue to establish the ability of the sediments to transform TCE and PCE. The current data indicate there is no apparent indigenous activity to transform TCE or PCE neither in the saturated soils of the phytoreactors at the site nor in the seepline soils. These microcosms are continuing and other potential seepline soil nutrient limitations (i.e. nitrogen) will be evaluated for bioremediation potential.

Soil Respiration. Table 4 shows the box or phytoreactor respiration rates. There was no significant box or treatment effect on soil metabolic rate. It is of interest that the oxygen consumption was higher in the deeper soils relative to the shallower soils in all boxes. This information demonstrated that while the bottom zone was saturated with groundwater throughout the project it was not completely anaerobic.

While the phytoreactor groundwater supply tank was filled from Well MSB 88 with consistent VOC concentrations (TCE 188 ppb, PCE 55 ppb), the phytoreactors actually receive groundwater through the supply system containing around 46 ppb TCE and 48 ppb PCE (Figures 7 & 8). These groundwater VOC losses within the system are most likely due to volatilization. While no statistically significant difference exists between boxes when comparing influent/effluent groundwater PCE/TCE concentrations, this evaluation did not take into consideration the overall water budget. As pointed out in the Results section, in comparing the groundwater influent flow rates from the three boxes (Figure 9)

to effluent flow rates (Figure 10). Box 2 with the poplars appeared to have the highest influent groundwater input and yet the lowest effluent output measured. This is not surprising since the poplar grew on the average over five feet during the year while the pine grew just over one foot. In addition the mass of roots from poplar is much larger and extensive in the boxes relative to the pine. The poplars did require more maintenance as it was attacked by insects twice requiring spraying. Groundwater flow through the boxes was monitored but inconsistent due to flow problems particularly in the soil control phytoreactor (Figures 3a-c). This box with no roots had greater settling of soil and associated packing of material. This soil control (Box 3) is being reconfigured to allow better flow. This next year, the groundwater influent and output will be monitored more stringently to better evaluate the contaminant removal.

Most compounds in soil (i.e. contaminants) must be in solution to be affected (absorbed, modified, degraded, sequestered, etc.) by either plants or microorganisms. Thus, water movement and nutrient availability in the rhizosphere is a critical factor as plants take up many times more water than is needed for metabolism and growth. This additional water is transpired through the leaves as the final step in nutrient transport. However, all of this water and compounds dissolved in it (the soil solution) moves through the rhizosphere, where it is subjected to processing by microorganisms before it enters the root. In some instances, the magnitude of microbial transformation of TCE can be significantly larger than plant transformation of TCE (Anderson and Walton, 1995). However, this is not always the case (Nichols et al., 1987; Schnabel et al., 1997). It is likely that both processes are useful in applying phytoremediation technology in TCE and PCE in this case for seepline groundwater remediation. Microbial activities to be assessed in FY01 will focus on the established rhizosphere activities relating to TCE/PCE removal.

Phytoremediation enjoys relatively favorable public acceptance, in part because it is perceived to be "natural" or non-intrusive. Essentially ambient process conditions and the lack of unsightly mechanical equipment also contribute to public acceptance. Use of genetically engineered plants has been found to be highly efficient for chlorinated ethene biodegradation (Doty et al., 2000), although this technology is not yet publicly acceptable. However, such plants are not needed at SRS (although they may offer process advantages at a later date when the acceptability issue has been resolved).

CONCLUSION

This project is highly significant in that most work in the phytoremediation area has been with much higher concentrations of VOC's (Burken and Schnoor, 1998, Newman et al, 1997, Doty et al., 2000). At SRS and other sites much of the VOC groundwater contamination with the exception of source areas is in lower (ppb) concentrations (WSRC-TR-00113 1999). The results of this project with concurrent SRS studies will enable better predictions of the VOC removal at the seepline. This first year, FY00 represented an initial acclimation phase for soil and plant systems and will facilitate examination of seepline phyto- and bioactivity in subsequent growth season in FY01. Initial results indicate that phytoremediation and MNA have considerable potential for the removal of TCE and PCE in the Tim's Branch flood plain and seepline.

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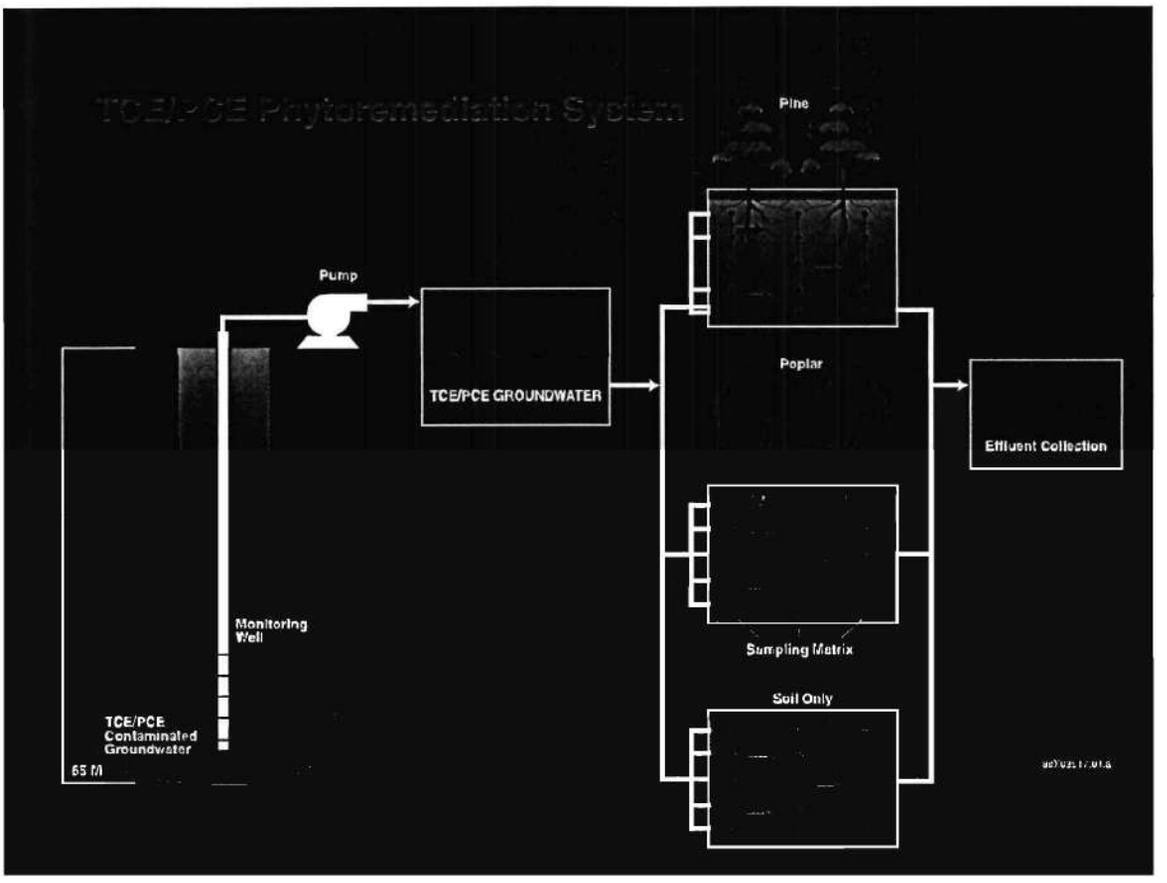

Figure 1. Phytoremediation System in Southern Sector of A/M area demonstrating groundwater supply system, three phytoreactors containing pine, poplar, soil control, and effluent collection system.

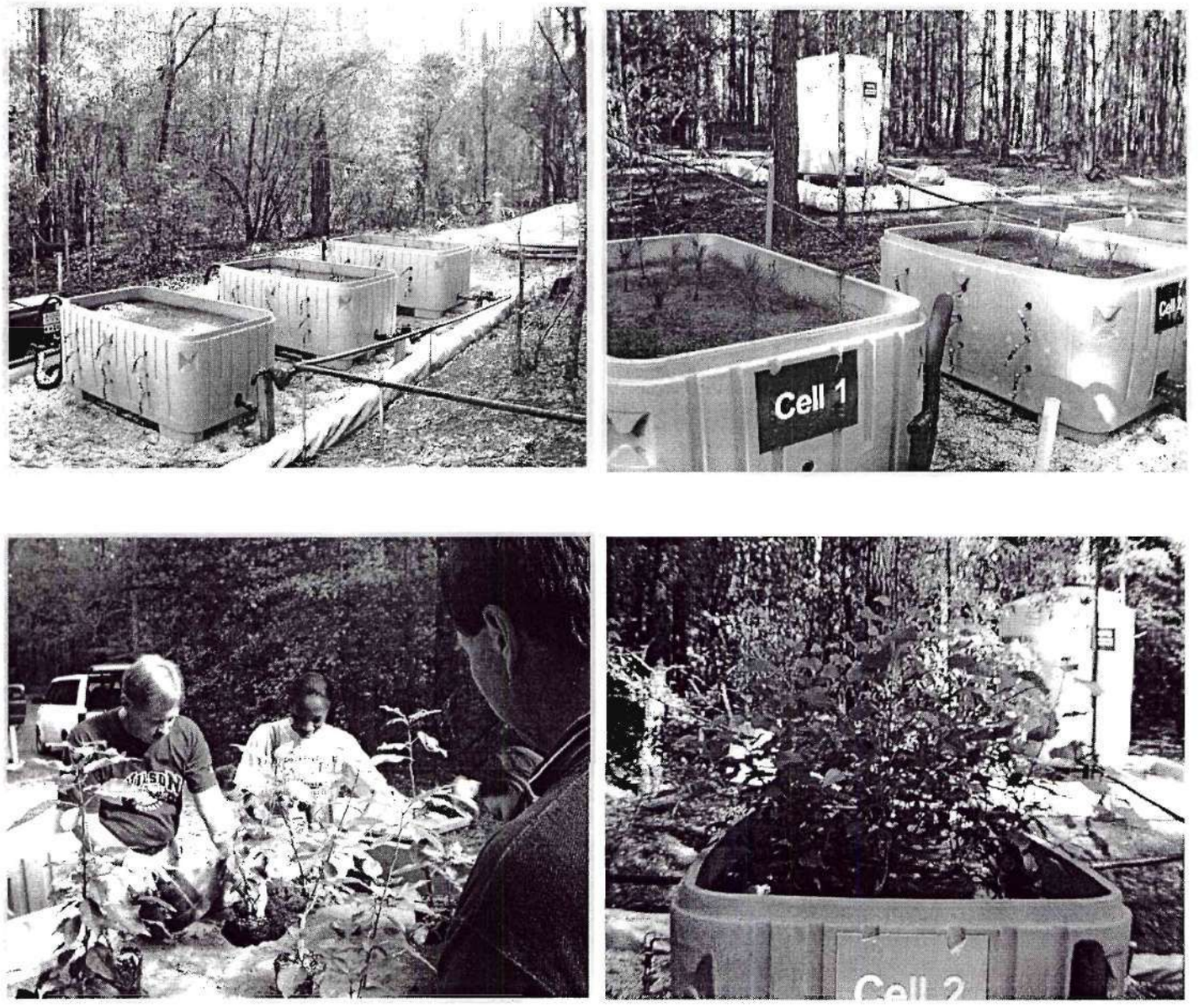

Figure 2. Setup of phytoreactors. Clockwise from upper left, phytoreactor boxes set up, site with groundwater supply tank in foreground, planting poplars, poplars in June 2000.

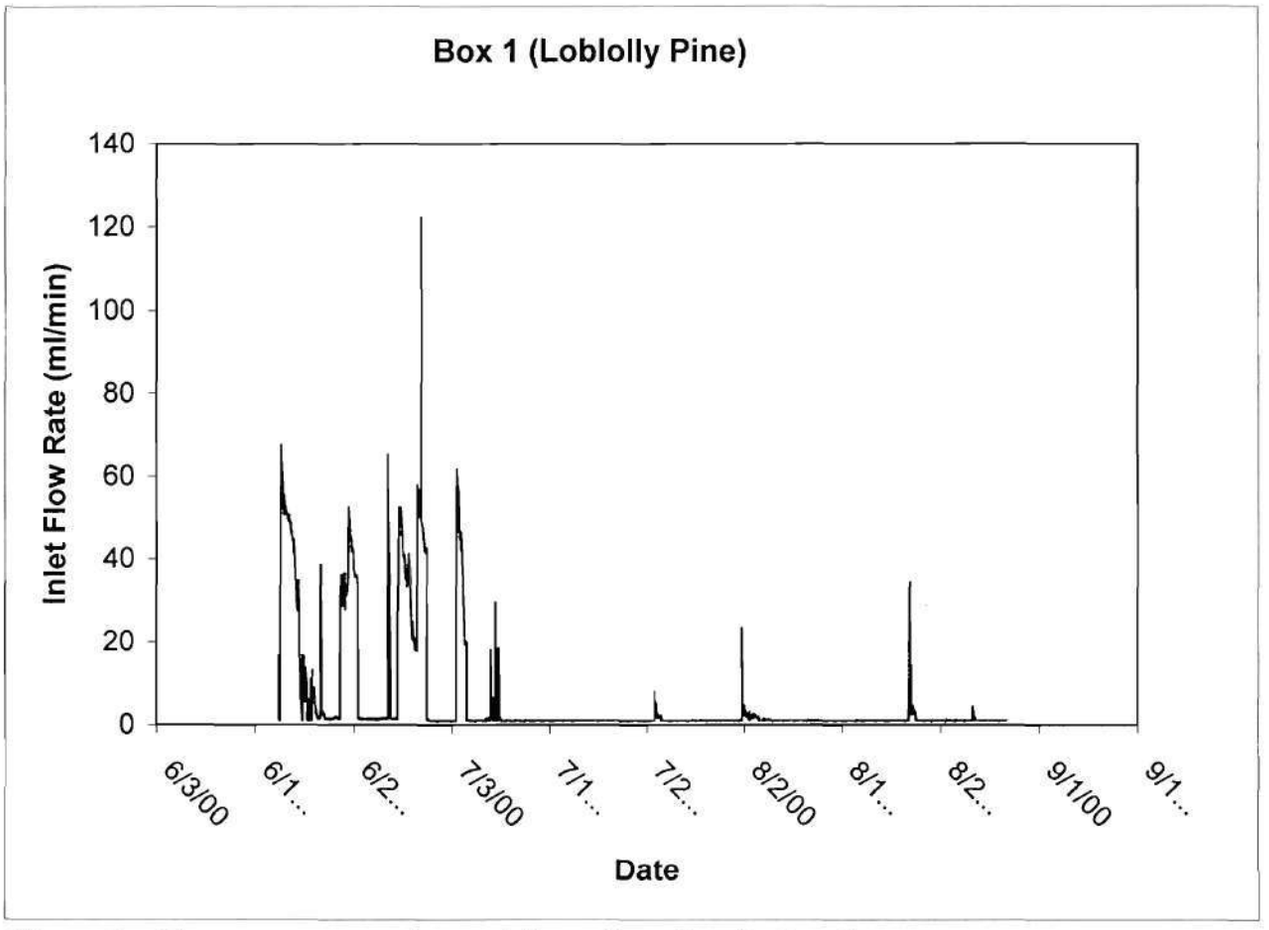

Figure 3a. Phytoreactor groundwater influent flow data for Box 1.

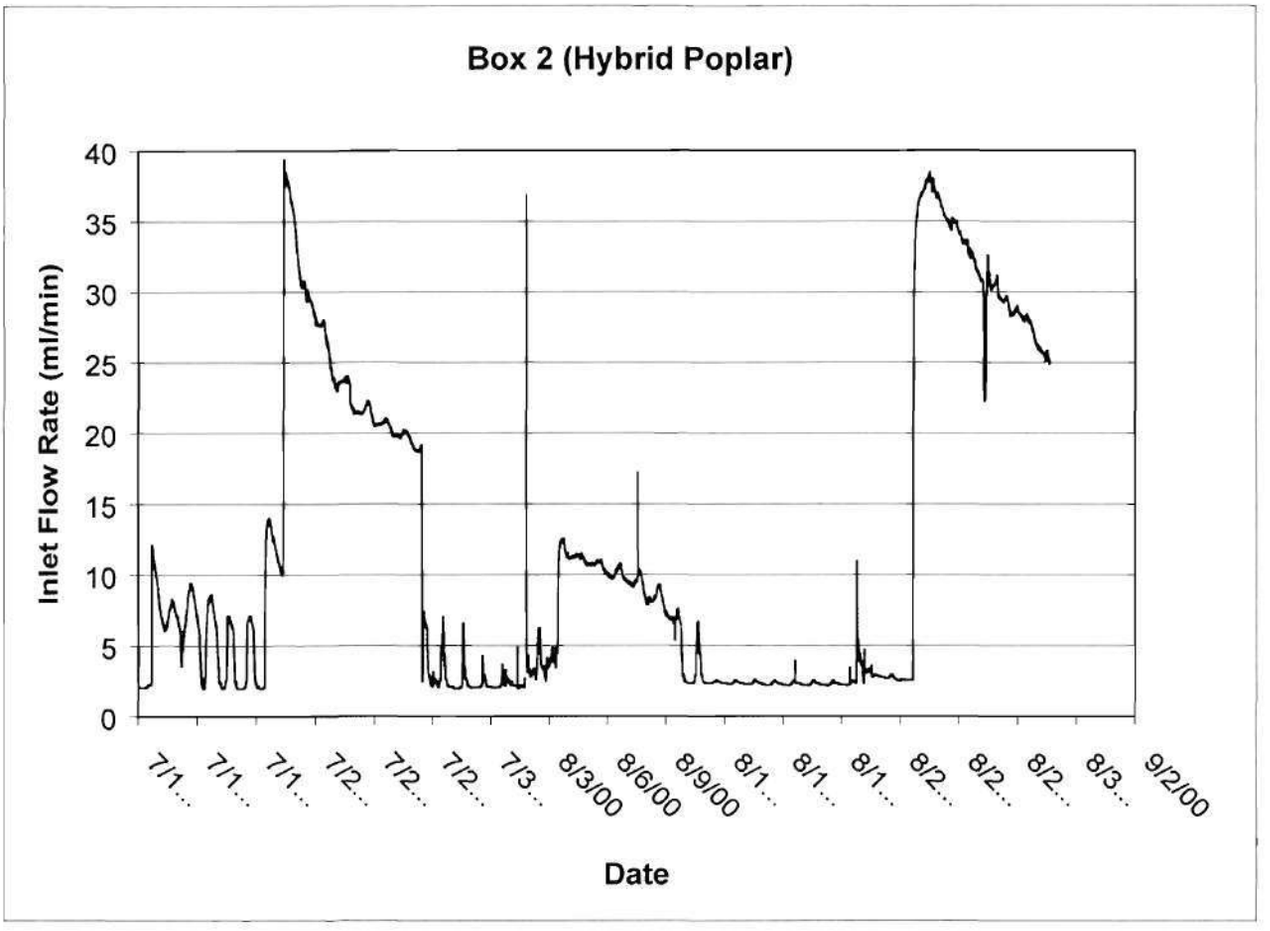

Figure 3b. Phytoreactor groundwater influent flow data for Box 2.

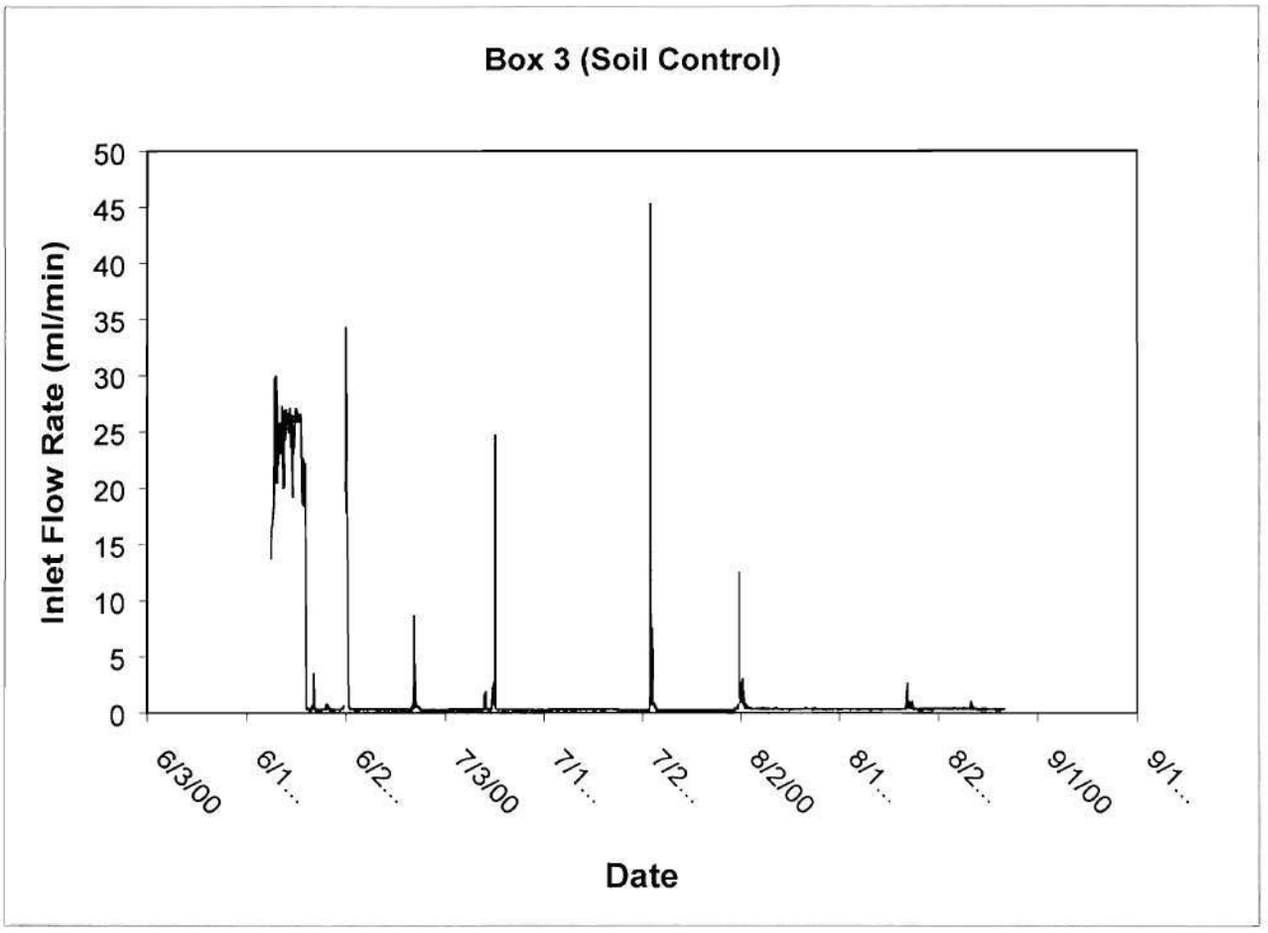

Figure 3c. Phytoreactor groundwater influent flow data for Box 1.

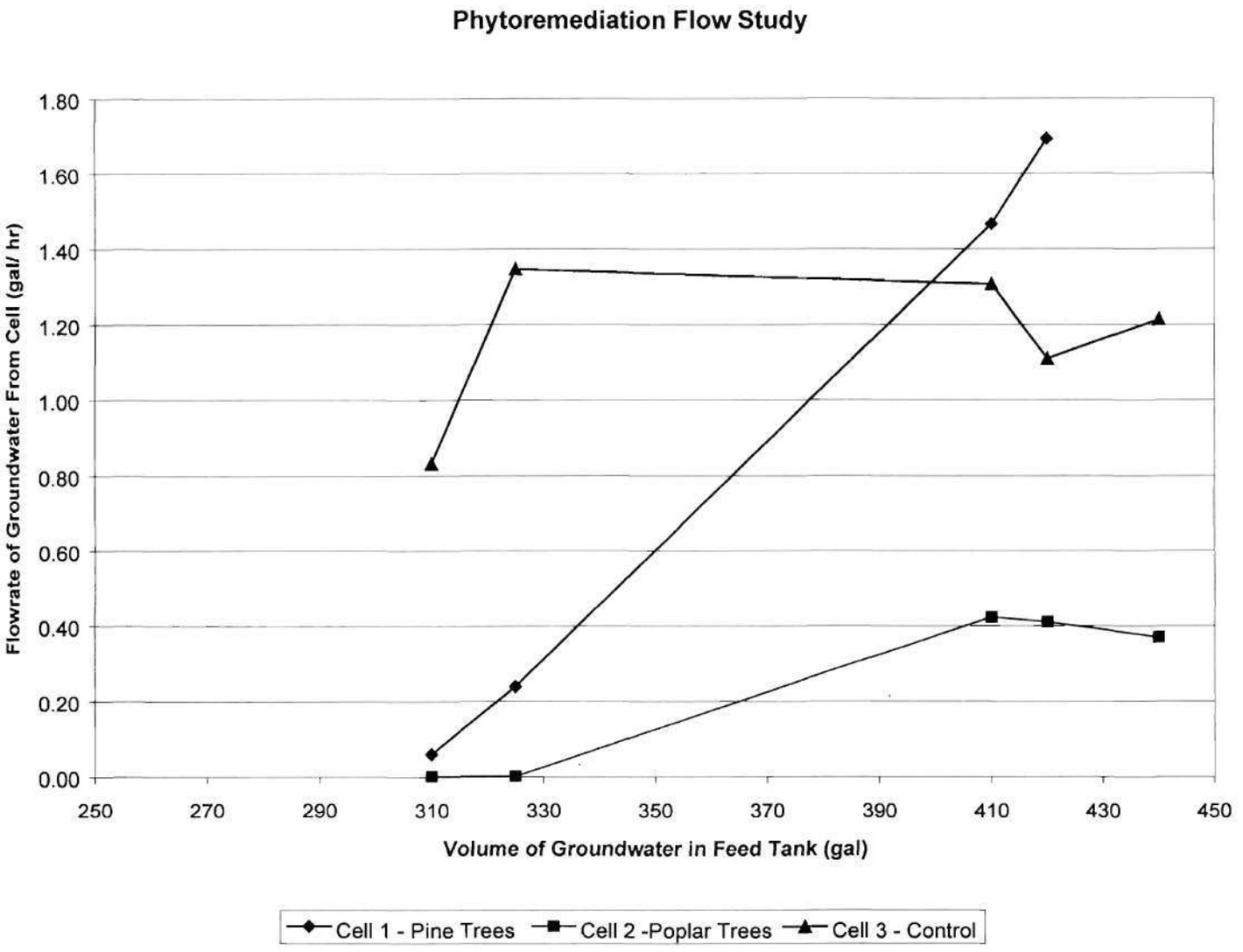

Figure 4. Phytoreactor groundwater effluent flow data for Boxes 1,2,and 3 during 8/9/00-8/17/00.

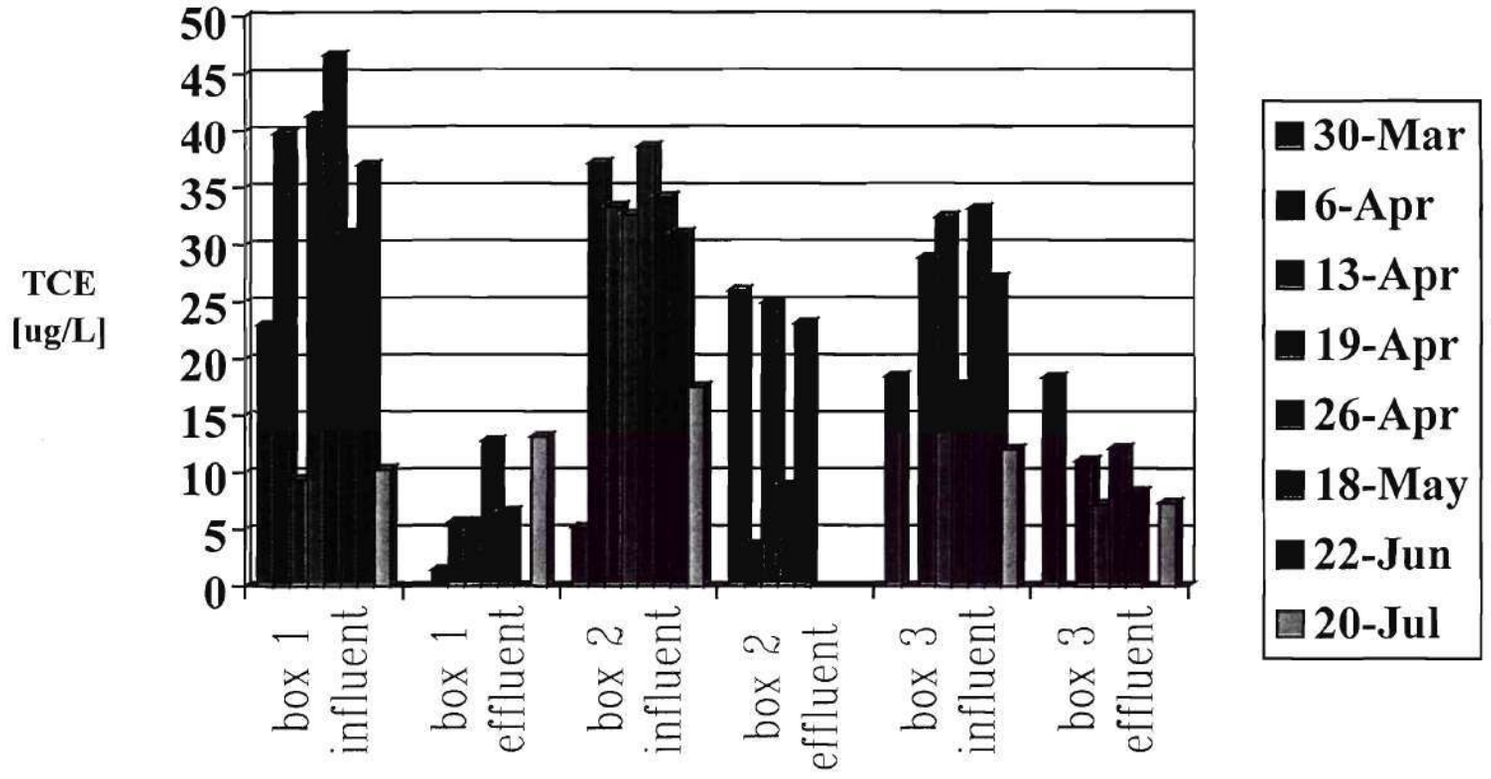

Figure 5. Phytoreactor groundwater influent and effluent TCE concentrations.

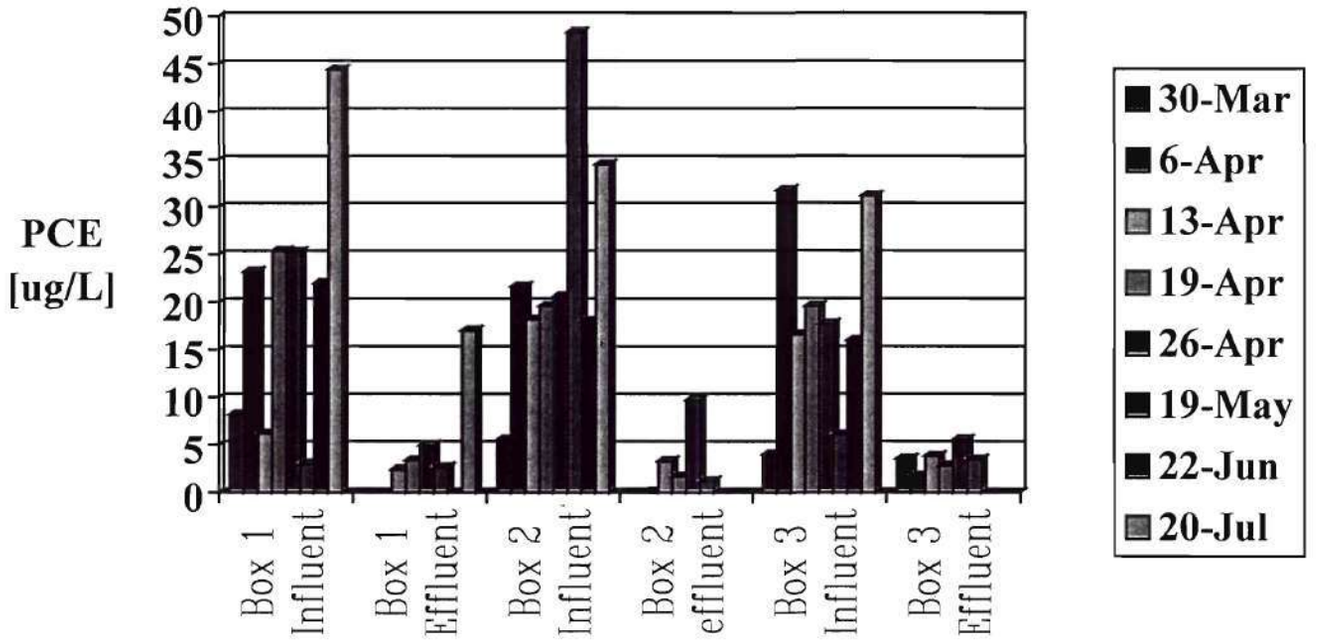

Figure 6. Phytoreactor groundwater influent and effluent PCE concentrations

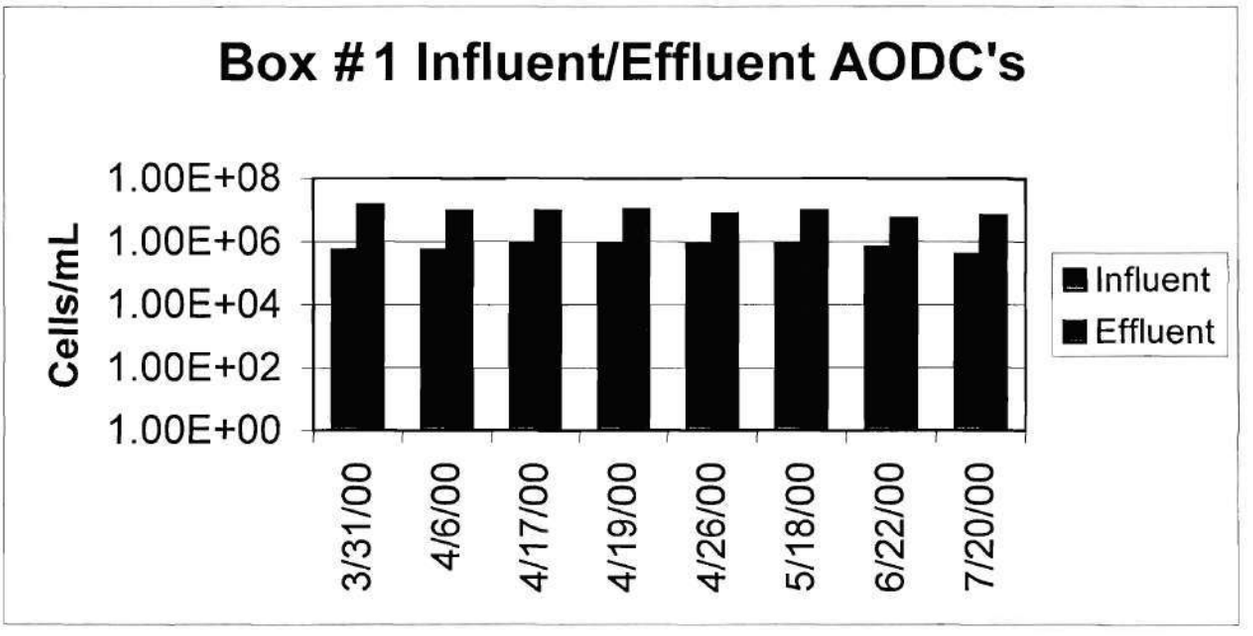

Figure 7a. Box 1 (Loblolly Pine) influent and effluent groundwater total bacteria/mL.

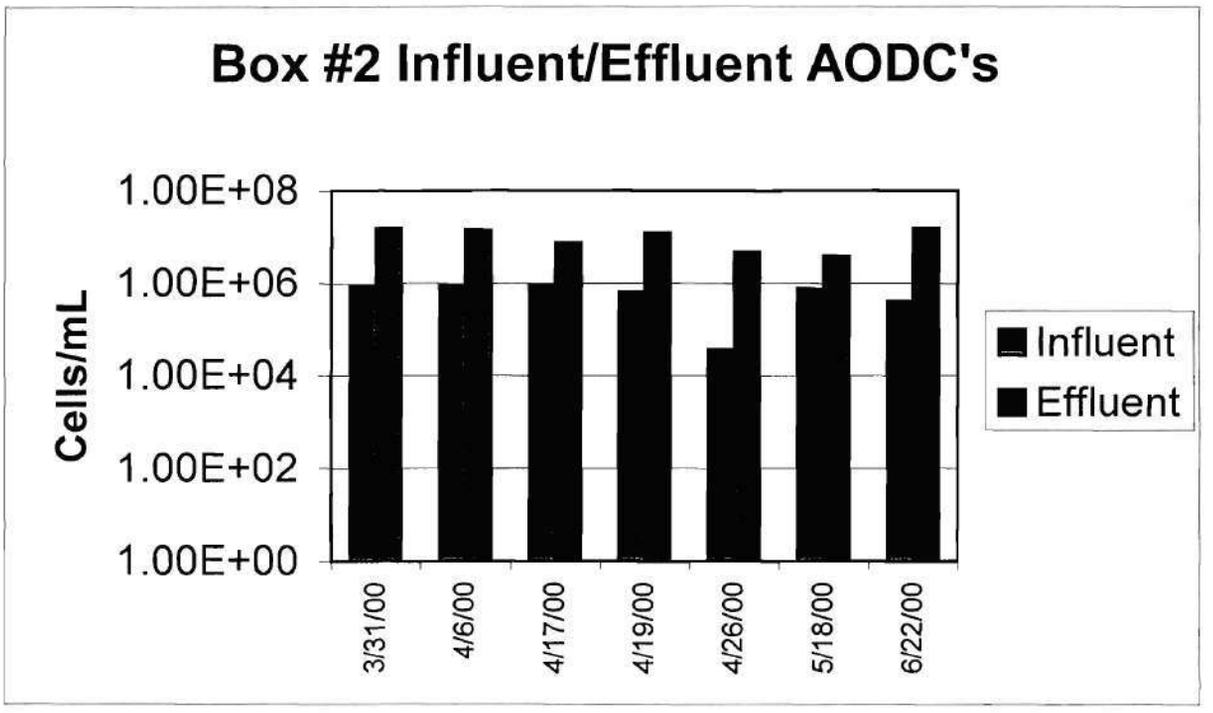

Figure 7b. Box 2 (Hybrid Poplar) influent and effluent groundwater total bacteria/mL.

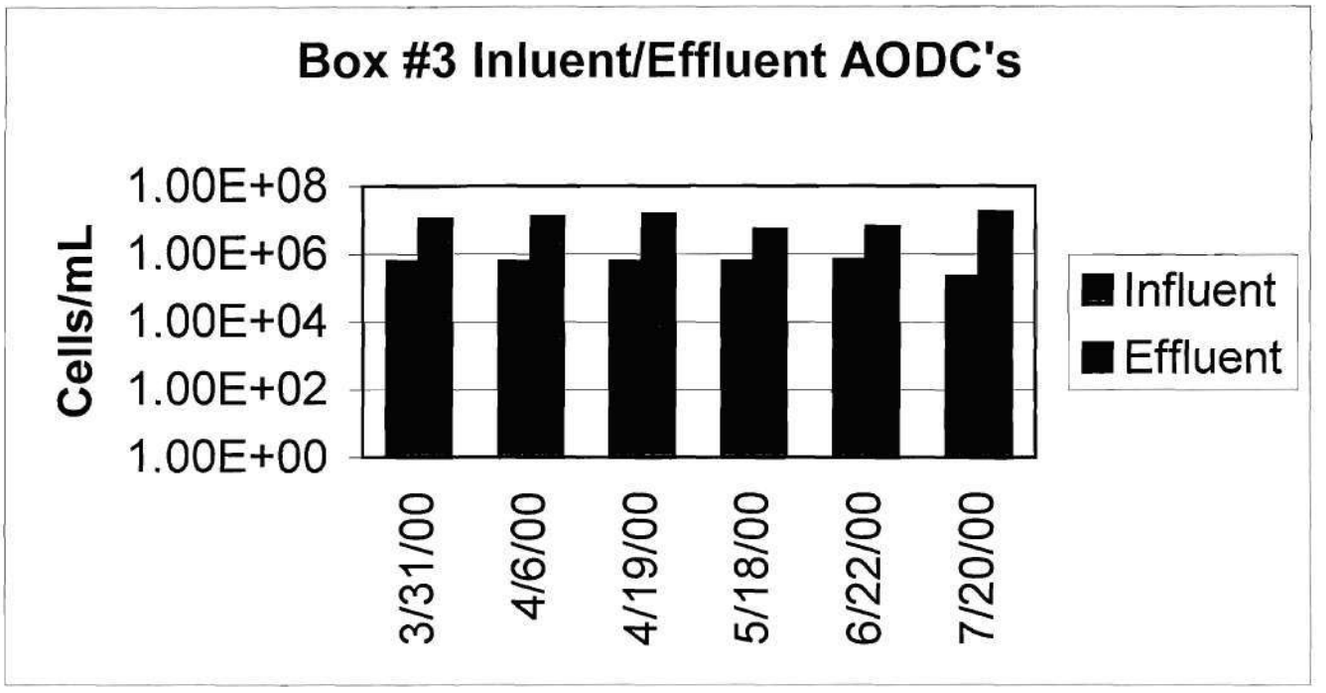

Figure 7c. Box 1 (Soil Control) influent and effluent groundwater total bacteria/mL.

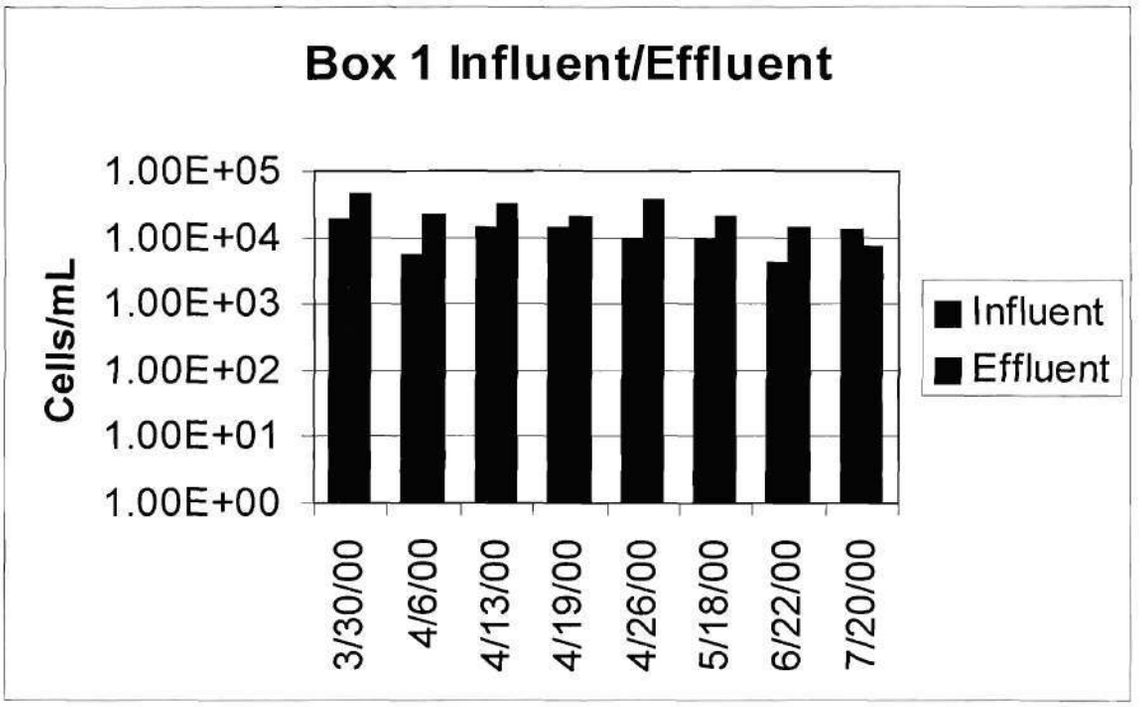

Figure 8a. Box 1 (Loblolly Pine) influent and effluent groundwater colony forming units/mL.

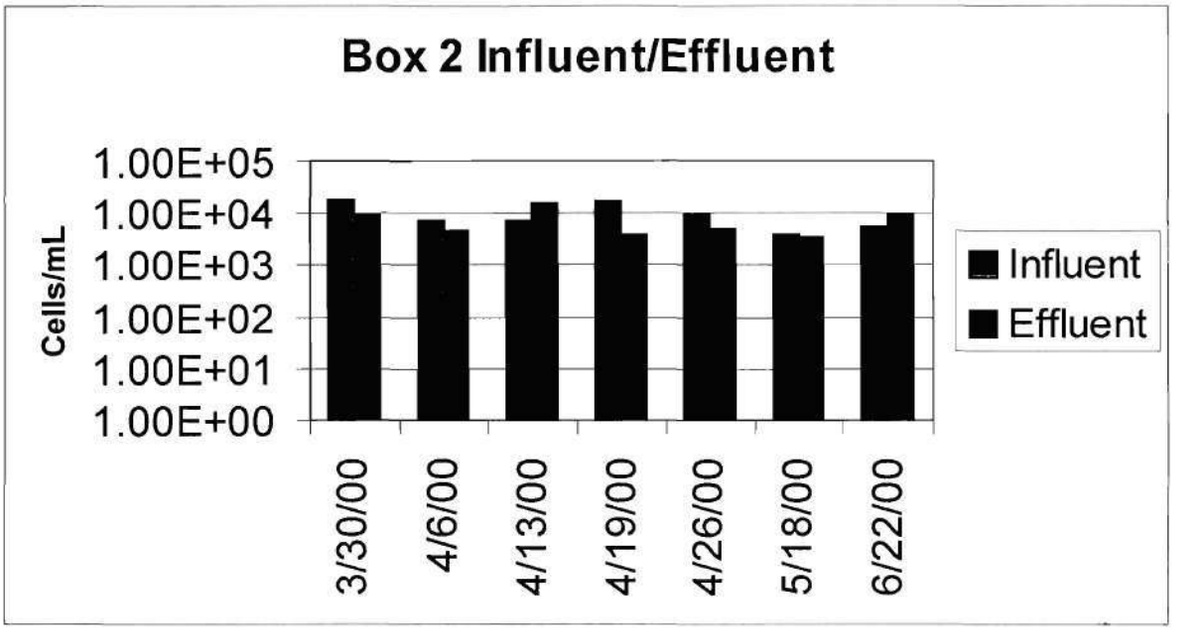

Figure 8b. Box 2 (Hybrid Poplars) influent and effluent groundwater colony forming units/mL.

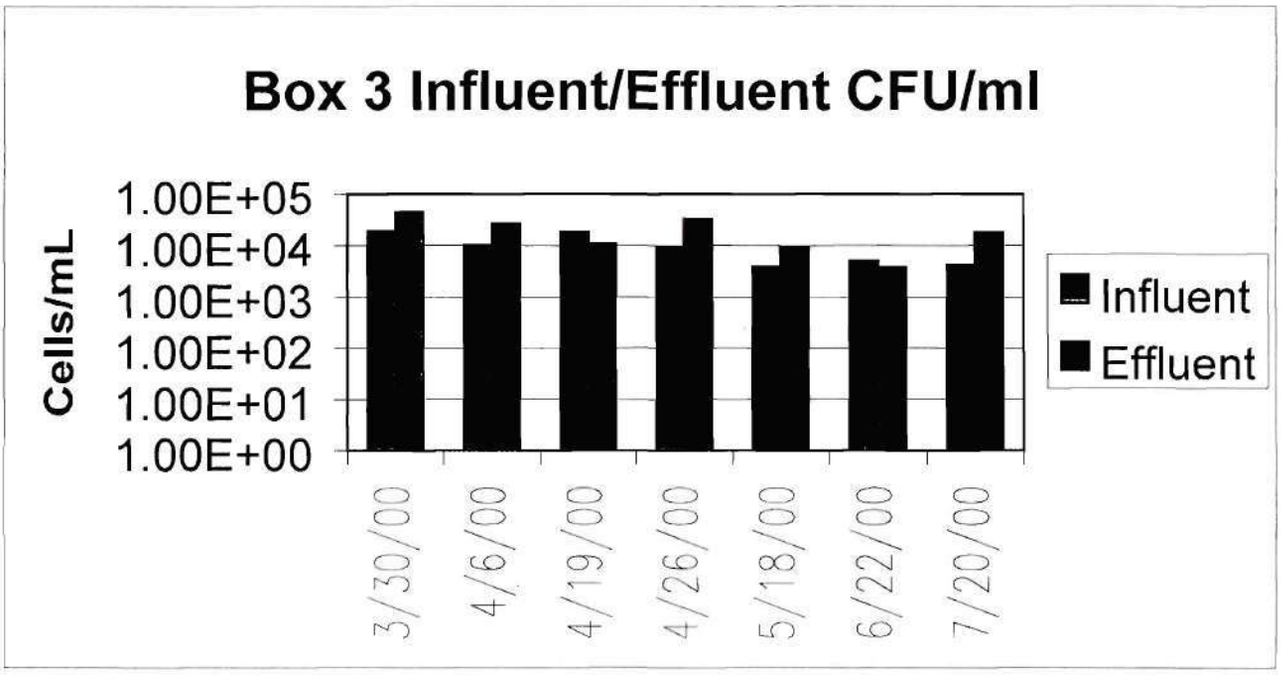

Figure 8c. Box 3 (Soil Control) influent and effluent groundwater colony forming units/mL.

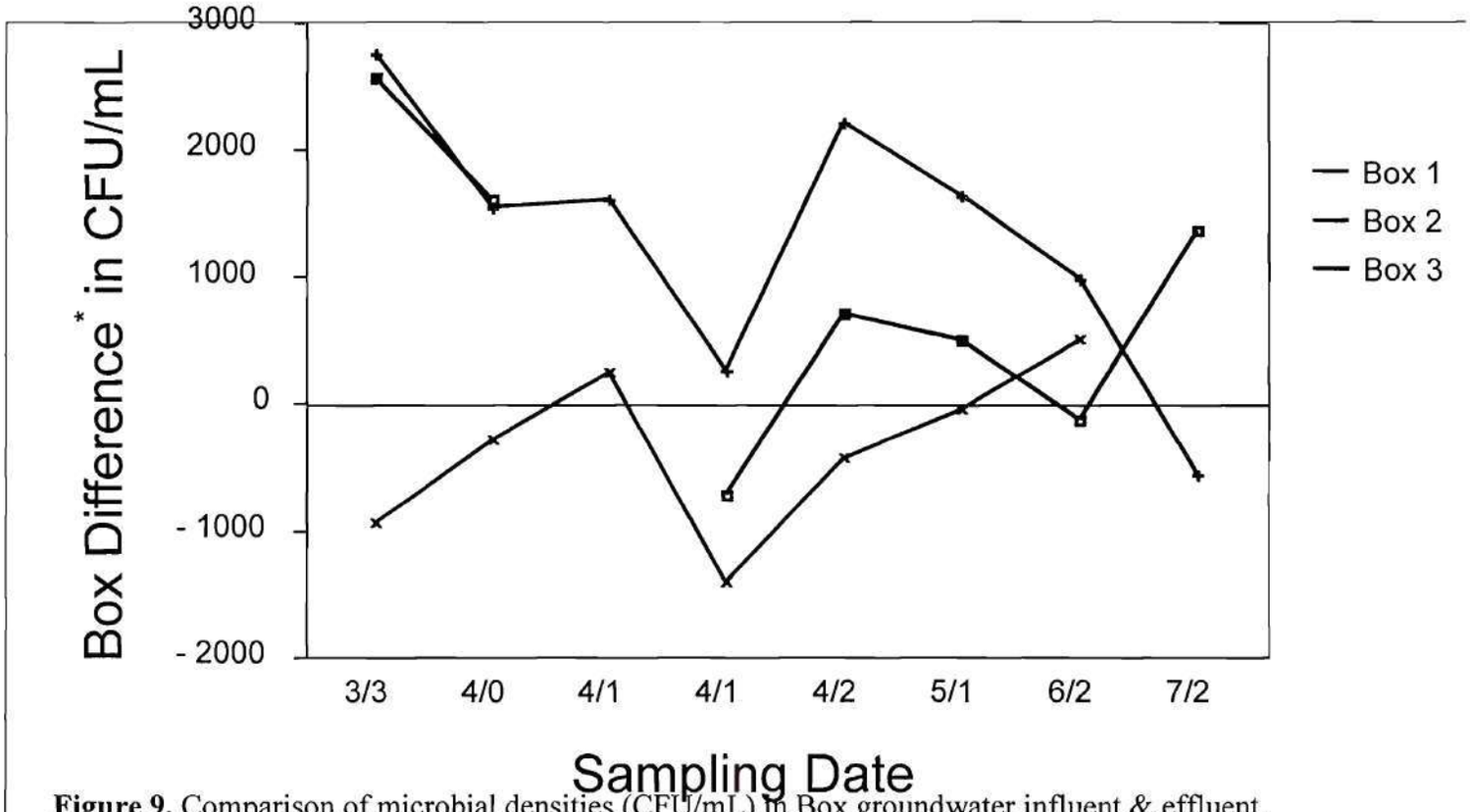

Figure 9. Comparison of microbial densities (CFU/mL) in Box groundwater influent & effluent.

- Difference = Effluent – Influent

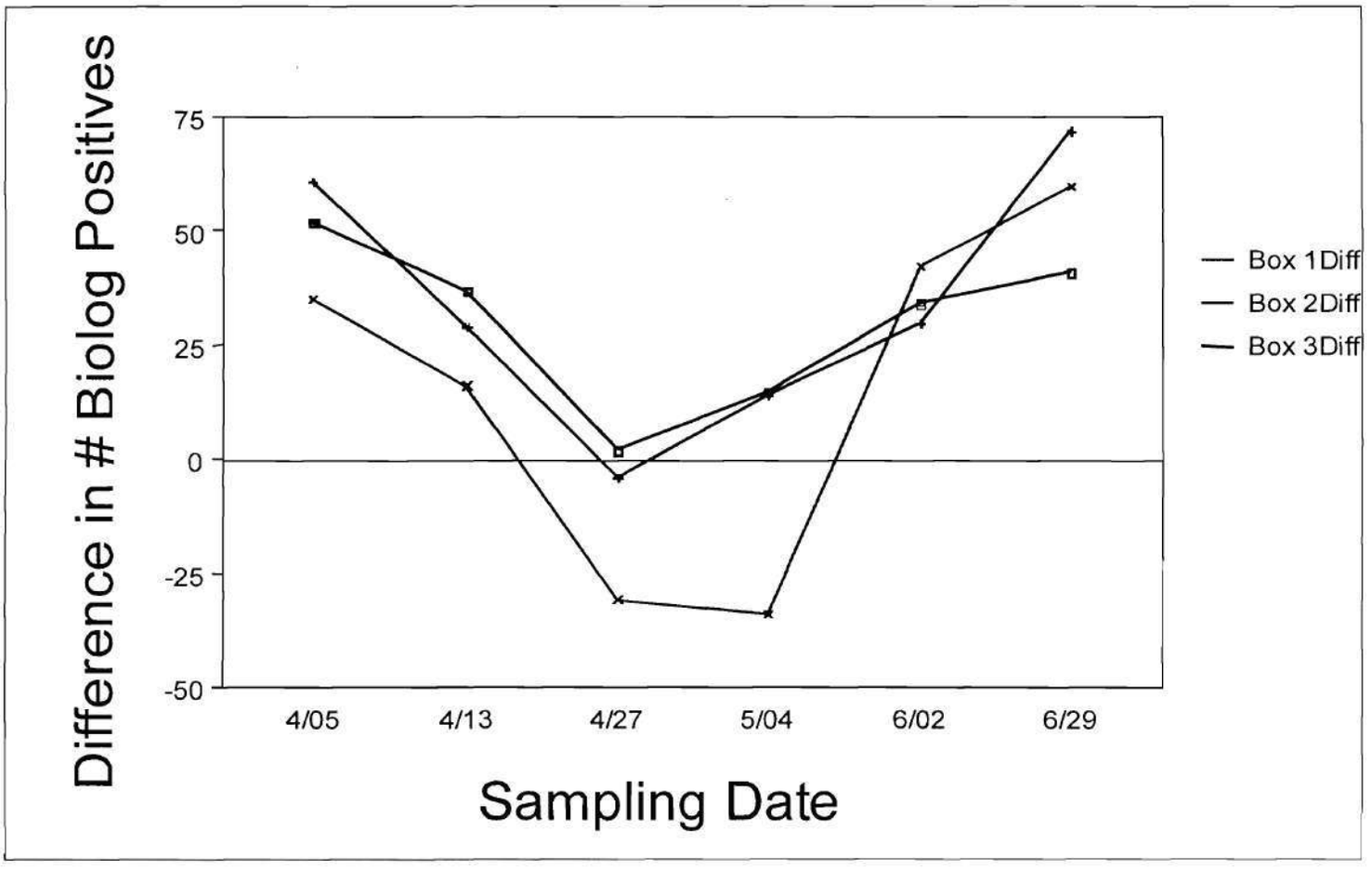

Figure 10. BIOLOG measurement of microbial diversity in the groundwater from the three boxes.

Table 1. Ion Chromatography of influent and effluent groundwater samples.

	Box #	Date	Chloride	Nitrite	Nitrate	Phosphate	Sulfate
Influent	2	30-Mar	2.93		3.80	2.44	0.86
Influent	1	6-Apr	5.97		4.04	11.57	0.95
Influent	2	6-Apr	3.23		5.47	1.39	0.79
Influent	3	6-Apr	3.23		5.63	0.70	0.76
Influent	1	14-Apr	3.19		4.92	<0.5	0.69
Influent	3	14-Apr	3.19		4.84	<0.5	0.76
Influent	2	26-Apr	3.34		6.07	<0.5	0.75
Influent	2	18-May	4.32		6.20		2.25
Influent	1	22-Jun	3.83		6.56		1.87
Influent	2	22-Jun	3.86		6.57		1.82
Influent	3	22-Jun	3.83		6.57		1.89
Influent	1	20-Jul	3.44		6.06	0.61	0.75
Influent	2	20-Jul	3.40		6.07	<0.5	0.75
Influent	3	20-Jul	3.45		6.08		0.76
Effluent	1	30-Mar	18.26		1.60	65.84	9.06
Effluent	2	30-Mar	22.32	<0.5	1.04	79.24	11.32
Effluent	3	30-Mar	38.76	<0.5	<0.5	144.68	7.66
Effluent	1	6-Apr	2.49		2.09	<0.5	7.08
Effluent	2	6-Apr	2.94	<0.5	2.08	<0.5	7.16
Effluent	3	6-Apr	2.81		1.93	<0.5	6.70
Effluent	1	14-Apr	2.15		0.98	<0.5	7.22
Effluent	2	14-Apr	3.08	<0.5	1.95	<0.5	5.72
Effluent	1	26-Apr	3.22		2.28	<0.5	4.44
Effluent	2	26-Apr	3.31	<0.5	4.55	<0.5	3.05
Effluent	3	26-Apr	3.19	<0.5	3.42	<0.5	4.52
Effluent	1	18-May	4.34	1.38	2.73		3.33
Effluent	2	18-May	4.26	1.51	4.86		2.43
Effluent	3	18-May	4.27	1.44	4.97	<0.5	3.28
Effluent	1	22-Jun	4.01				7.59
Effluent	2	22-Jun	4.00	1.28	4.33		2.11
Effluent	3	22-Jun	6.21				11.24
Effluent	1	20-Jul	3.67		<0.5		1.58
Effluent	3	20-Jul	5.02	<0.5	0.91		15.61

Table 2. Microcosms Established with SED 1 and SED 2

Electron Donor	Electron acceptor	Replicates
Acetate	PCE	3
Acetate	TCE	3
Acetate	cis-DCE	3
Acetate	VC	3
Acetate*	No electron acceptor	1
H_2	PCE	3
H_2	TCE	3
H_2	cis-DCE	3
H_2	VC	3
H_2*	No electron acceptor	1
Killed/H_2*	PCE	2
Killed/H_2*	TCE	2
Killed/H_2*	cis-DCE	2
No Electron donor*	PCE	3
No Electron donor*	TCE	3
No Electron donor*	cis-DCE	3
No Electron donor*	VC	3
Lactate	PCE	3
Lactate	TCE	3
Lactate	cis-DCE	3
Lactate	VC	3
Lactate*	No electron acceptor	1
Lactate	1,2-Dichloropropane	1
Lactate	1,2,3-Trichloropropane	1
Lactate	1,1,1-Trichloroethane	1

*** Controls**

Table 3. Methanogenic activity in Microcosms for SED 1 and 2

Microcosm	Methane peak height
PCE lactate SED1	140
PCE acetate SED1	36
PCE H_2 SED1	120
PCE no e^- donor SED1	28
PCE kill 1	0
PCE kill 2	0
TCE lactate SED1	185
TCE acetate SED1	39
TCE H_2 SED1	0
TCE H_2 SED2	0
TCE no e^- donor SED1	50
TCE kill 1	0
TCE kill 2	0

Table 4. Phytoreactor soil metabolic rates.

Chamber Number	SS Box number	O2 ul/gram dry weight	Std Dev O2/ gram dry weight	CO2 ul/gram dry weight	Std Dev CO2/ gram dry weight
1	control	N/A	N/A	N/A	N/A
2	Box 1 shallow	1.40 e-5	2.176 e-5	7.98 e-6	1.904 e-5
3	Box 1 deep	7.087e-6	1.1060 e-5	4.342 e-6	9.580 e-6
4	Box 2 shallow	1.192 e-5	2.041 e-6	3.395 e-5	8.027 e-6
5	Box 2 deep	7.134 e-6	1.221 e-5	3.026 e-6	7.724 e-6
6	Box 3 shallow	1.252 e-5	2.301 e-5	6.663 e-6	1.679 e-5
7	Box 3 deep	6.348 e-6	1.022 e-5	4.147 e-6	1.031 e-5